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BIREFRINGENCE SIGNALS FROM SURFACE AND T-SYSTEM MEMBRANES OF FROG SINGLE MUSCLE FIBRES

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SUMMARY

1. When the tonicity of Ringer is increased above 2·5 times normal and a single fibre stimulated externally, the large, early birefringence signal preceding twitch tension (Baylor & Oetliker, 1975, 1977*a*, *b*) is sufficiently reduced and delayed so as to reveal a small but distinct signal ('1st component') preceding it. For an average-sized fibre, the $\Delta I/I$ of the 1st component was (minus) 1 to 2×10^{-5}.

2. The time course of the 1st component superimposed with the surface action potential simultaneously recorded by an internal micro-electrode. The polarity of the 1st component reversed with compensation.

3. From these characteristics, the 1st component is thought to arise from a small change in optical retardation of the surface membrane due to the action potential.

4. When a fibre was impaled with two micro-electrodes, retardation changes accompanying small hyperpolarizing and depolarizing current steps were detected. In some cases, the polarity of the observed signal was opposite in sign to that expected for a retardation change only from the surface membrane.

5. Because the anatomical orientation of the T-system appears to be primarily transverse rather than longitudinal, these signals of opposite polarity are probably, on balance, due to retardation changes from the membranes of the T-system.

6. The possible origin of the large birefringence signal preceding contraction is discussed.

INTRODUCTION

The two preceding papers (Baylor & Oetliker, 1977*a*, *b*) have described and analysed some of the physiological and optical properties of a large birefringence signal from skeletal muscle ($\Delta I/I$ in the range of 10^{-3}),

occurring just after the surface action potential but before the development of twitch tension. The properties of this signal make it likely that it reflects some step taking place within the fibre volume just preceding, or perhaps associated with, activation of the contractile elements.

The experiments of this paper describe two other birefringence signals that can be detected from single muscle fibres. These signals are closely related to the electrical activity of the surface and T-system membranes. Because of the much smaller magnitude of these signals ($\Delta I/I$ in the range of 10^{-5}), their detection required the use of signal-averaging and experimental situations in which the much larger signal mentioned above was either absent or greatly reduced in size. On balance, one of these signals appears to reflect primarily potential change across the surface membrane while the other signal may reflect potential change across the membranes of the T-system.

In view of these and the preceding results (Baylor & Oetliker, 1975, 1977*a*, *b*), the possible origin of the large birefringence signal is discussed. An explanation consistent with its known properties is that this signal reflects a potential change across the membranes of the sarcoplasmic reticulum associated with the release of Ca^{2+}. However, other explanations may also be possible.

A brief account of some of the results has previously been published (Baylor & Oetliker, 1975).

METHODS

Most of the methods were as described previously (Baylor & Oetliker, 1977*a*). Experiments using micro-electrodes were done with the Reichert polarizing microscope. The electrodes were filled with 3 M-KCl for voltage recording or 2 M-K citrate for current passing and had resistances between 3 and 20 MΩ. For the simultaneous electrical and optical measurements, a fibre was impaled in the middle of the field of optical illumination with either one or two electrodes. When two electrodes were used, the impalements were made on diametrically opposite sides of the fibre.

RESULTS

The '1st component' and the surface action potential

The large birefringence signal preceding twitch tension has been shown to propagate away from the stimulating cathode with a speed corresponding to that expected for the action potential (Baylor & Oetliker, 1977*a*, Figs. 7 and 8). From the birefringence experiments on the squid giant axon (Cohen, Hille & Keynes, 1970), one would expect that the earliest part of this propagating signal in muscle would be a birefringence signal from the surface membrane due to the action potential. Judging from the size of the 'optical spike' in squid, however, one could not expect to detect such a signal from a single muscle fibre without signal-averaging.

When the earliest optical change was examined near the stimulating cathode by signal-averaging in a large number of single fibres in normal Ringer, at least 90 % showed no evidence of a distinguishable hump or shoulder in the rising phase of the optical signal. The usual finding was that the light intensity changed smoothly into the large, early signal preceding the rise of tension. A few fibres in normal Ringer did show the suggestion of a small shoulder, although never a distinct peak, as the

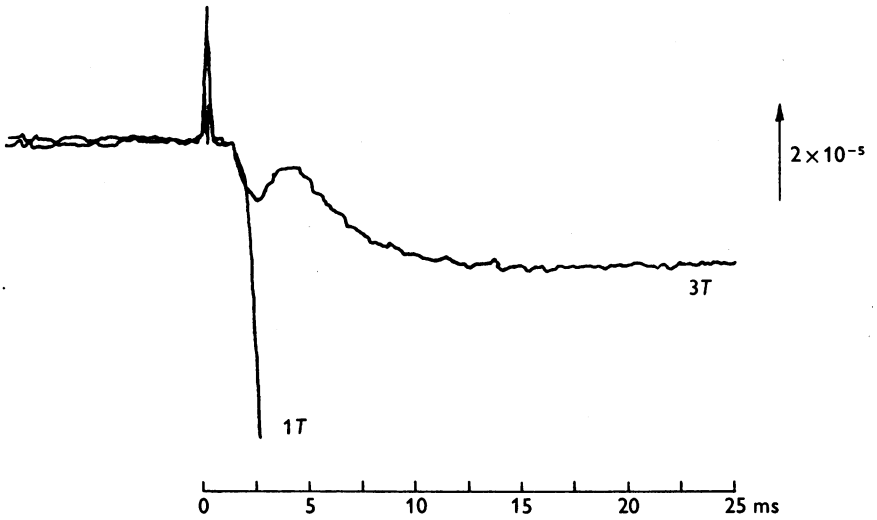

Fig. 1. The effect of increasing tonicity on the earliest take-off of the optical signal. $1T$ = 120 mM-NaCl Ringer; $3T$ = 370 mM-NaCl Ringer. Each trace shown is the average of 128 sweeps in the solution indicated. Moment of stimulation (zero time) is indicated by the large stimulus artifact. Muscle unrecorded; stretch unrecorded; white light; 1·5 mm from cathode; AC coupled; room temperature; fibre ref. 053174.02; viewing width of fibre (μm) and R_{max} (nm) was 160 μm and 187 nm in $1T$ Ringer and 128 μm and 202 nm in $3T$ Ringer. For the $3T$ trace, the early signal (time-to-peak 3 ms following stimulation) is called the '1st component' and the more slowly developing signal (time-to-peak about 15 ms) is called the '2nd component'.

earliest signal recorded near the cathode. Evidently in isotonic Ringer the large, early optical signal reflecting events subsequent to excitation of the surface membrane obscures the peak of the action potential signal that quite likely is present. Similarly inconclusive results were obtained when signal-averaging was used in an attempt to detect the 'optical action potential' from fibres bathed in D_2O Ringer. However, for fibres bathed in $2{\cdot}2T$ hypertonic Ringer an early signal, with time course similar to that expected for the surface action potential, was usually seen with signal-averaging. Furthermore, as the tonicity was raised beyond $2{\cdot}2T$, this signal was progressively revealed (Baylor & Oetliker, 1975).

An example of this effect is shown in Fig. 1. Signal-averaged records of the earliest take-off of the optical signal were recorded near the cathode in $1T$ and $3T$ Ringer. In isotonic Ringer the optical signal departs rapidly from the base line, showing no sign of a small, early component. In $3T$ Ringer, however, a distinct early signal is revealed ('1st component'), preceding a greatly reduced and delayed '2nd component'. Allowing for a conduction velocity of 2 mm/ms, the time to peak of the 1st component in $3T$ Ringer is about 1 ms, appropriate for the surface action potential at room temperature. Since the initial rising phases of the $1T$ and $3T$ signals superimpose, it is reasonable to conclude that an intensity change corresponding to the 1st component is also present in normal Ringer but that its peak is obscured by the rapidly rising 2nd component.

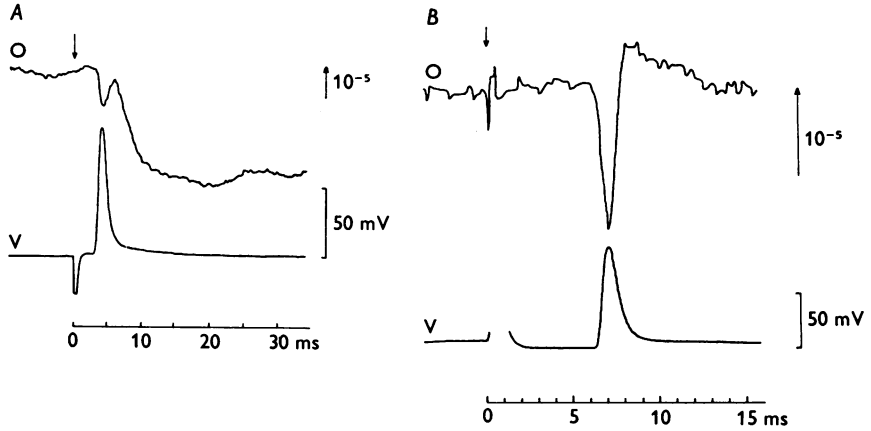

Fig. 2. Timing of the 1st component and the surface action potential. For both parts of the Figure, the optical signal (O) and action potential recorded by internal micro-electrode (V) were averaged simultaneously for a total of 300 sweeps in $3T$ Ringer at room temperature. *A*, muscle, and stretch were not recorded; fibre width was 65 μm; R_{max}, 216 nm; white light; 7 mm from cathode; optical AC and voltage DC coupled; fibre ref. 060674.02. Resting potential varied between -70 and -65 mV. *B*, muscle, stretch, and distance from cathode were not recorded; fibre viewing width 168 μm; white light; both optical and voltage signals were AC coupled; fibre ref. 070274.01. Resting potential varied between -90 and -85 mV.

If the 1st component is not an artifact, one would expect to see it propagate over the fibre length. This property was observed, with the measured conduction velocity of the 1st component in $2{\cdot}8T$ Ringer close to that expected for the action potential (2 mm/ms at 20° C).

To clearly establish the timing of the 1st component relative to the surface action potential, it was necessary to use an internal micro-electrode to record the action potential directly. Results from two experiments are shown in Fig. 2. In each case, the fibre was impaled with a single

voltage recording electrode in the middle of the area of optical illumination and the optical and intracellular potential signals were simultaneously averaged for a total of 300 sweeps. In both cases the 1st component (trace O) and the action potential (trace V) have nearly superimposable time courses until late in the falling phase of the action potential. Since this result from muscle is very similar to that found in the squid giant axon (Cohen *et al.* 1970), it seems reasonable to attribute most of the 1st component to the potential change across the surface membrane. Another point of interest in Fig. 2*B* is the virtual lack of any 2nd component of the optical signal. This is a dramatic example of the finding that the 2nd component is greatly reduced in strongly hypertonic Ringer compared with normal Ringer (Baylor & Oetliker, 1975, 1977*a*).

In Fig. 2*B*, the 'falling' phase of the 1st component actually crosses the base line to become a net intensity increase compared with resting. A similar phenomenon was seen for the birefringence signal from squid, in that the light signal 'undershot' more than the action potential (Cohen *et al.* 1970). Thus, whatever the explanation is for this phenomenon, it may be the same in muscle as in nerve. However, an alternate explanation in muscle is also possible (see *Birefringence signals from the T-system*, below).

If the 1st component in muscle is caused by the same mechanism which produces the optical spike in nerve, the 1st component should also behave as a small change in optical retardation ('ΔR hypothesis'). Because of the small size of the signal, and the fact that a fibre in 3*T* Ringer will give at best only about 300 action potentials, it was impractical to perform the complete set of tests of the ΔR hypothesis, as was done in squid (Cohen *et al.* 1970) and for the large, early birefringence signal in muscle (Baylor & Oetliker, 1977*b*). However, the compensation experiment shown in Fig. 3 makes it likely that the optical mechanism underlying the 1st component is a small change in retardation. The fibre was illuminated with white light in order to maximize the signal-to-noise ratio. Trace O_2 shows the 1st component in the uncompensated fibre and trace O_1 when -288 nm of retardation ($\frac{1}{2}$ wave-length at $\lambda = 575$ nm) was introduced by the compensator. The 1st component clearly inverted with compensation, as is confirmed by trace $S/2$, the sum of O_1 and O_2 divided by 2, which is flat through the time to peak of the 1st component. Thus the 1st component also appears to be explainable as a change in retardation.

Size of the first component retardation change

On the assumption that the 1st component reflects a change in retardation of the surface membrane, it is of interest to calculate the size of the retardation change normalized with respect to a single thickness of active membrane (see, for example, Cohen *et al.* 1970: Baylor & Oetliker, 1977*b*).

This 'membrane parameter', denoted $\Delta R_{surface}$, can be calculated knowing the characteristics of the illuminating radiation, the resting retardation of the fibre, the size of the observed $\Delta I/I$ of the signal, and assumptions regarding the quantity and disposition of the active membrane giving rise to the signal.

Table 1 gives the results of this calculation for the peak $\Delta R_{surface}$ of the

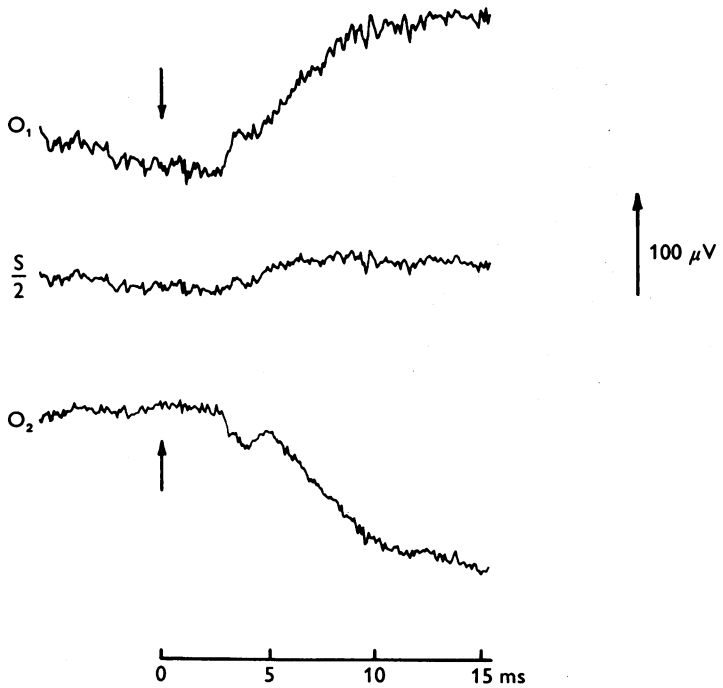

Fig. 3. Reversal of 1st component with compensation in 2·75T Ringer. Optical records were taken with −288 nm (O_1) or with 0 nm (O_2) of retardation introduced by a compensator. Sixteen sweeps were averaged for trace O_1 and 24 sweeps for O_2. Another 32 sweeps with −288 nm of retardation taken after O_2 also showed the 1st component to be an increase in light intensity but with some deterioration compared with O_1. Iliofibularis m.; fibre diameter, R_{max}, and distance from cathode were not recorded; stretch, 1·73 times slack length; white light; DC coupled; temp. 22° C; fibre ref. 072775.03. Resting light intensity for trace O_2 was 1·29 V.

1st component for fibres in Ringer solutions between 2·3 and 3 times hypertonic. The average magnitude for $\Delta R_{surface}$ is $2{\cdot}15 \times 10^{-3}$ nm (twelve fibres). The same polarity of the retardation change (a decrease) was observed in all fibres examined. The direction of the change is therefore the same as observed in axons (Cohen *et al.* 1970; Von Muralt, 1975).

In contrast to the calculations for the large, early signal seen in normal Ringer or D_2O, which is thought to be volume-related (Baylor & Oetliker, 1977*b*) the 1st component retardation change was calculated under the assumption that it arises from a structure in or near a longitudinally oriented surface membrane with a radially oriented optic axis (see Cohen *et al.* 1970). The exact formula for calculating $\Delta R_{surface}$ is specified by eqns. (19), (20) and (23) of Baylor & Oetliker (1977*b*).

TABLE 1. Estimation of $\Delta R_{surface}$ for first component signal in strongly hypertonic solutions

Fibre ref. (1)	Stretch (%) (2)	Relative tonicity (T) (3)	R_{max} (nm) (4)	$R_{\frac{1}{4}}/R_{\frac{3}{4}}$ (% of R_{max}) (5)	$-\Delta I$(peak) I ($\times 10^{-5}$) (6)	$-\Delta R_{surface}$ ($\times 10^{-3}$ nm) (7)
053174.02	—	3	202	—	1·70	2·69
121174.01	157	2·8	288	89/89	1·40	3·39
070274.01	—	3	187	86/86	1·63	2·37
112774.03	131	2·75	259	74/74	0·59	1·25
072275.02	142	2·3	158	75/75	2·50	3·04
060174.01	—	2·5	216	—	0·84	1·44
060674.02	—	3	216	—	1·20	2·06
070174.02	—	3	216	89/89	1·60	2·74
070274.02	—	3	173	58/92	0·88	1·18
071774.01	170	2·9	202	100/64	1·50	2·38
091274.02	—	2·7	187	85/85	1·20	1·74
121874.02	170	2·5	223	90/90	0·85	1·51
Average ± S.E. of mean						2·15 ± 0·20 (n = 12)

All fibres were illuminated with white light. Dashes in table indicate data were unrecorded. Only those fibres whose 1st components showed a distinct peak (as in Figs. 1–3) are included in Table 1. This criterion includes nearly all fibres in Ringer more hypertonic than 2·5T for which sufficient signal-averaging was done. See text for assumptions used in calculating the values in column 7.

The assumption that the angular disposition of the membrane giving rise to the signal is a smooth right circular cylinder is probably at best only a rough approximation, since a muscle fibre in 3T Ringer shrinks to about 56 % of its normal volume and the surface membrane wrinkles and folds (Blinks, 1965). In particular, the exterior membrane of such a shrunken fibre should contain about one third more area than is accounted for by this assumption and a significant fraction of the membrane may be at a different angle to the light path than is predicted by a smooth cylindrical contour. In experiments using a small slit of light incrementally moved across the fibre width, attempts were made to verify the spatial distribution of the 1st component signal assumed in the calculations, i.e. maximal at the edges and zero in the middle (Cohen *et al.* 1970). Due to the small size of the signal and the limited number of action potentials that can be elicited from a fibre in 3T Ringer, these experiments were unsuccessful.

Another potential source of error in the calculation of $\Delta R_{surface}$ is retardation changes from the 'caveolae', the numerous small invaginations of the surface membrane (Rayns, Simpson & Bertaud, 1968; Franzini-Armstrong, 1973). However, since the caveolae are nearly spherical (Zampighi, Vergara & Ramon, 1975; Dulhunty &

Franzini-Armstrong, 1975) and are optically in series with a large resting retardation, their net contribution to the intensity change should be essentially zero. Any contribution to ΔI from one part of the sphere should be equal and opposite to the same part of the sphere rotated 90° about the direction of the light path.

Birefringence signals from the T-system

Morphological evidence indicates that the T-system membranes in frog twitch fibres are, on balance, oriented in the transverse direction, whereas the surface membrane is longitudinally disposed (Peachey, 1965; Peachey & Schild, 1968; Mobley & Eisenberg, 1975). Since both membrane systems are in series with the same large resting retardation, one would expect depolarization of the T-system to produce a signal opposite in sign to that from a surface depolarization, i.e. a positive rather than negative ΔR.

A signal of opposite polarity to the 1st component, possibly due to tubular depolarization, is seen in Fig. 2*B*, where an intensity increase reaches a peak about 1·5 ms after the 1st component. The occurrence of such a signal was atypical, however, perhaps because the 2nd component decrease in retardation normally obscures any retardation increase from the T-system. The chances for detecting a retardation change from the T-system might be considerably improved if voltage displacements across the tubular membranes were imposed but kept below the contractile threshold.

Fig. 4 shows results from such an experiment in normal Ringer on a fibre illuminated with white light. The fibre was impaled in the middle of the field of illumination with two micro-electrodes, one for passing current and the other for recording potential. The voltage and optical signals were recorded and averaged simultaneously in response to small hyperpolarizing and depolarizing current steps of relatively long duration (part *A*) and relatively short duration (part *B*). Small intensity changes are apparent in all optical traces, with a time course which closely follows the change in surface membrane potential. Since in normal Ringer the potential of the T-system can be expected to rapidly follow the surface potential (Adrian, Chandler & Hodgkin, 1969, Appendix), the optical signals in Fig. 4 also closely follow the change of the T-system potential. The sign of the light intensity changes in this and other similar experiments could be reversed by adding an appropriate amount of compensation (trace O_4, Fig. 4*B*), making it likely that the underlying optical mechanism is a change in retardation.

The relationship between the sign of the intensity changes and the sign of the voltage changes observed in Fig. 4 is of interest, however. From the measured maximum retardation of this fibre after insertion of the micro-electrodes (288 nm) and the theory discussed on pages 21–22 of Baylor & Oetliker (1977*b*) taking into account the use of white light, an increase in intensity in the uncompensated records corresponds to a net increase in

retardation, whether the structure giving rise to the signal is distributed as surface or volume. The intensity changes observed in Fig. 4 are therefore in the wrong direction to be consistent with a retardation signal from a

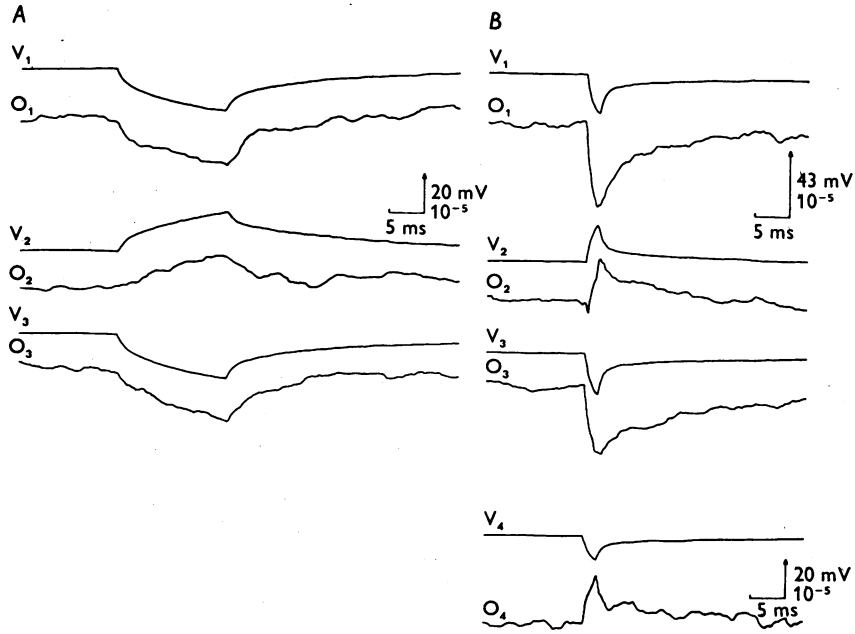

Fig. 4. Two micro-electrode current-passing experiment, showing the simultaneously recorded voltage (V) and optical (O) responses during small depolarizing and hyperpolarizing current steps of relatively long duration (15 ms, part *A*) and relatively short duration (1·5 ms, part *B*). Average fibre diameter and stretch not recorded. An R_{max} of 288 nm was measured after insertion of the two diametrically opposed internal micro-electrodes. Normal Ringer; white light; optical signal AC coupled, voltage DC coupled; room temperature; fibre ref. 061174.02. All optical traces were uncompensated, except trace O_4, which had -140 nm of retardation introduced by the compensator. Traces O_4 and V_4 and all records in part *A* are the average of 640 sweeps; the remaining records in part *B* are the average of 1024 sweeps. During the experiment, the viewing width of the fibre was approximately 200 μm. Resting potential of the fibre after impalements was -90 mV. No optical signal was detected with the voltage electrode inside the cell and the current electrode just outside the cell. In part *B*, upper calibration bar applies to tracing pairs 1–3.

longitudinally disposed surface membrane (Cohen *et al.* 1970; Von Muralt, 1975; Table 1, this paper).

Retardation increases accompanying small depolarizations were observed in other similar current-passing experiments in both normal and hypertonic Ringer. However, this finding was not consistently observed.

Of ten such experiments small depolarizations were accompanied by an increase in retardation in four fibres and a decrease in retardation in six fibres.

DISCUSSION

The experiments of this paper demonstrate that changes in optical retardation can be detected in single muscle fibres accompanying voltage changes across the surface and T-system membranes. If the mechanism underlying these changes in muscle is similar to that occurring in nerve, the direction of the retardation change contributed by an area of membrane should be predictable from the orientation of the membrane with respect to the resting retardation, and the direction of the associated potential change. This seems to be the case for the 1st component, which appears to be attributable to the surface action potential.

In contrast, the polarity of the retardation change accompanying small voltage displacements of surface and T-system membranes around the resting potential (e.g. Fig. 4) was not found to have the same relationship to the direction of the potential change in all experiments. A possible explanation for this phenomenon in terms of the net orientation of the membrane systems involved is suggested by the morphological data of Mobley & Eisenberg (1975). Their stereological analysis of membrane orientation indicated that T-system membranes were nearly isotropically disposed, the average net orientation being 17 % in the transverse direction. Thus 83 % of a retardation signal from the T-system might be expected to cancel and the remainder should give rise to a positive rather than a negative ΔR with depolarization. The polarity of the combined signal from surface and T-system membranes might therefore depend on fibre diameter, since in a small fibre the signal from the longitudinal surface membrane could outwegh a signal of opposite sign from the T-tubular membranes. Another possibility is that the net orientation of the T-system membranes might vary with stretch, perhaps even becoming longitudinal in a highly stretched fibre. In the latter case, a T-tubular signal should add to, rather than subtract from, the surface signal. Unfortunately, not enough current-passing experiments were successfully completed to decide on the likelihood of these speculations.

Possible origin of the large birefringence signal preceding contraction

With the results available on birefringence signals from surface and T-system membranes, the large birefringence signal preceding contraction (Baylor & Oetliker, 1975, 1977*a*, *b*) will now be discussed. Since this signal appears to be due to a change in retardation distributed as fibre volume,

its possible origin will be considered in terms of what volume-related structures are likely to undergo a retardation change within the first few milliseconds after stimulation.

T-system. The magnitude (Baylor & Oetliker, 1975) and in some cases polarity (this paper) of the retardation changes observed during current-passing experiments make it unlikely that the large birefringence signal preceding contraction can be explained in terms of tubular depolarization alone. Rather, the results support the idea that this large optical signal is regulated in a highly non-linear way by depolarization of surface and T-system membranes into the contractile range (Baylor & Oetliker, 1975). Further investigation into the mechanism of this regulation may be more favourably pursued in voltage-clamp experiments.

Triadic junction. It is generally accepted that excitation–contraction coupling in frog twitch fibres involves structural changes at the level of the triads, where one transverse tubule is in close contact with two adjacent membranes of the sarcoplasmic reticulum. Franzini-Armstrong (1970) has described a regular array of particles ('feet') bridging the gap separating the cytoplasmic leaflets of the T-system and adjacent membranes of the sarcoplasmic reticulum. It has been suggested that these particles are closely related to the site of the voltage-dependent charge movement in skeletal muscle that may serve to directly regulate Ca^{2+} release (Schneider & Chandler, 1973). One simple hypothesis along these lines might be that in response to the T-system potential change the early change in retardation reflects movement of these charged molecules or portions of these molecules extending, perhaps through the feet, to the adjacent sacroplasmic membrane. Although the kinetic features of the charge movement argue against this possibility (Chandler, Rakowski & Schneider, 1976), no direct evidence exists either in favour of or against this or any similar hypothesis. Because of the early timing of the birefringence signal, the possibility should be kept in mind that its origin may be localized in the region of the triadic junction.

Sarcoplasmic reticulum. It has been speculated previously that the early retardation change may reflect a potential change across the membranes of the sarcoplasmic reticulum associated with the release of Ca^{2+} (Baylor & Oetliker, 1975). This explanation is attractive because (1) the time course of the signal is compatible with an event related to Ca^{2+} release, (2) it is reasonable to suppose that Ca^{2+} release may normally be accompanied by a sizeable sarcoplasmic reticulum potential change, and (3) the retardation change accompanying the action potential in squid (Cohen *et al.* 1970) and electrical activity of surface and T-system membranes in muscle (Baylor & Oetliker, 1975, and this paper) appears to be explained by a membrane potential change. Although the net orientation of the sarcoplasmic reticulum

membrane like the surface membrane is longitudinal (Peachey, 1965; Mobley & Eisenberg, 1975), it is not known if the direction of the large early retardation change is correct for signalling sarcoplasmic reticulum potential change. This uncertainty remains even if it is assumed that the myoplasmic potential becomes positive with respect to the inside of the sarcoplasmic reticulum as Ca^{2+} is released, since it is unclear which side of the membrane of the sarcoplasmic reticulum should be identified with which side of the surface membrane for predicting the polarity of the retardation change.

Additional evidence in favour of the idea that there is a significant potential change in the sarcoplasmic reticulum at the time of Ca^{2+} release has been obtained from fluorescent dye experiments. Bezanilla & Horowicz (1974, 1975) have detected a large, early change in extrinsic fluorescence in whole frog muscle stained with Nile Blue A, a dye which has been shown in nerve to give a relatively large fluorescence change in response to a membrane potential change (Cohen, Salzberg, Davila, Ross, Landowne, Waggoner & Wang, 1974). The characteristics of this dye signal in muscle are remarkably similar to many of the features described for the large, early birefringence signal, including its propagating nature, approximate time course, and response to depolarizing currents, nitrate, and D_2O (Bezanilla & Horowicz, 1975). These authors have suggested that this fluorescence signal arises from a membrane potential change of the sarcoplasmic reticulum.

Experiments measuring simultaneous birefringence and fluorescence signals on the same single fibre are also of interest (Oetliker, Baylor & Chandler, 1975). In these experiments the fluorescence signal from an indodicarbocyanine dye, also shown in nerve to give a relatively large signal in response to membrane potential change (Cohen *et al.* 1974), was compared with the retardation change simultaneously measured in hypertonic Ringer. Through the time to peak, the two signals were nearly superimposable, supporting the conclusion that they arise from the same underlying process.

Along these lines, it is of interest to compare the magnitude of the ΔR of the various retardation signals that have been suggested to arise from a membrane potential change. Values of ΔR normalized to a single thickness of membrane (Cohen *et al.* 1970) are given in Table 2 for several different preparations. Since the three corresponding potential changes in Table 2 are all about 100 mV, the values for the normalized ΔRs are perhaps more remarkable for their differences than their similarity. It is not clear how much of this variation is to be attributed to true differences in the underlying signals and how much to errors in the assumptions underlying the calculations. For example, because of uncertainties in the quantity and

disposition of the membranes assumed to give rise to the signals in muscle (Baylor & Oetliker, 1977*b*, and this paper), the figures for muscle could easily be in error by a factor of 2.

Some of the variation in the normalized ΔR in Table 2 may, however, be real. In voltage-clamp experiments on the squid giant axon over a wide potential range, ΔR was shown to be approximately a parabolic, not linear

TABLE 2. Estimates of normalized ΔR presumed to arise from a membrane potential change

Presumed membrane system	Peak ΔR (single thickness) ($\times 10^{-3}$ nm)	Corresponding potential change (mV)
(1)	(2)	(3)
Squid giant axon[1]	$-0{\cdot}145$	100
Rabbit vagus axons[2]	$-1{\cdot}0$	100
Frog surface membrane[3]	$-2{\cdot}2$	90*
Frog SR membrane[4]	$-5{\cdot}9$	?

[1] Cohen *et al.* (1970).

[2] Calculated from unpublished data of L. B. Cohen, R. D. Keynes & J. M. Ritchie.

[3] Table 1, this paper.

[4] Calculated from Baylor & Oetliker (1977*b*), Table 5 and eqn. (30), for fibres in normal Ringer. Because of the occurrence of mechanical activity at the time of the measurement, this peak ΔR may involve more than one process. The corresponding figure for highly stretched fibres in D_2O, where the mechanical response has been nearly eliminated, is $-2{\cdot}4 \times 10^{-3}$ nm.

* Action potential measured in 3*T* Ringer.

function of potential (Cohen, Hille, Keynes, Landowne & Rojas, 1971). A different ΔR for the same potential change may therefore be due to a different resting condition. Another possible source of variation in Table 2 might be that signals arising from structures distributed in a repeating and closely spaced geometry (such as rabbit vagus nerve or sarcoplasmic reticulum membrane) are larger because of a greater contribution from 'form' rather than 'intrinsic' birefringence in comparison with the surface-related signals. A third possibility is that the structure of the membranes in question may be significantly different.

If one assumed that the conditions giving rise to the surface and sarcoplasmic reticulum signals in muscle are similar, it would be tempting to use the surface signal as a calibration to convert the reticulum retardation change to a voltage change. In view of the many uncertainties mentioned above, this step would necessarily be quite speculative without additional information. It would seem reasonable to conclude, however, that the sarcoplasmic reticulum potential hypothesis, if correct, would require a substantial mV change to explain a ΔR_{SR} larger than $\Delta R_{surface}$.

Myoplasm. Specific explanations of the ΔR involving myoplasmic changes are difficult to construct, but this possibility has not been ruled out. As an example, the movement of Ca^{2+} ions from inside the sarcoplasmic reticulum into the myoplasm might be associated with rapid volume changes of myoplasm relative to other structures (Sandow, 1966). This in turn could produce a change in form birefringence detectable as a change in retardation.

Contractile proteins. Since myosin accounts for the large birefringence of the A bands (Huxley & Hanson, 1957) and actin is also somewhat birefringent (Colby, 1971), contractile protein activity may be considered a likely source for giving rise to a retardation change. Changes in contractile protein retardation probably explain the large, more slowly developing birefringence signal accompanying tension (Von Muralt, 1932, 1975; Bozler & Cottrell, 1937; Eberstein & Rosenfalck, 1963). When contractile activity was varied by either stretch or mechanically deactivating solutions (Baylor & Oetliker, 1977*a*), the late birefringence signal accompanying tension provided a good monitor of this activity. A similar explanation for the large, early birefringence signal may also be possible. For example, an early conformational change in troponin due to the binding of Ca^{2+}, or a subsequent change in tropomyosin, might explain the large retardation change preceding tension.

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REFERENCES

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1969). The kinetics of mechanical activation in frog muscle. *J. Physiol.* **204**, 207–230.

BAYLOR, S. M. & OETLIKER, H. (1975). Birefringence experiments on isolated skeletal muscle fibres suggest a possible signal from the sarcoplasmic reticulum. *Nature, Lond.* **253**, 97–101.

BAYLOR, S. M. & OETLIKER, H. (1977*a*). A large birefringence signal preceding contraction in single twitch fibres of the frog. *J. Physiol.* **264**, 141–162.

BAYLOR, S. M. & OETLIKER, H. (1977*b*). The optical properties of birefringence signals from single muscle fibres. *J. Physiol.* **264**, 163–198.

BEZANILLA, F. & HOROWICZ, P. (1974). Fluorescence changes in frog muscle stained with Nile Blue associated with excitation-contraction coupling. *Fedn Proc.* **33**, 1259.

BEZANILLA, F. & HOROWICZ, P. (1975). Fluorescence intensity changes associated with contractile activation in frog muscle stained with Nile Blue A. *J. Physiol.* **246**, 709–735.

BLINKS, J. R. (1965). Influence of osmotic strength on cross-section and volume of isolated single muscle fibres. *J. Physiol.* **177**, 42–57.

BOZLER, E. & COTTRELL, C. L. (1937). The birefringence of muscle and its variation during contraction. *J. cell. comp. Physiol.* **10**, 165–182.

CHANDLER, W. K., RAKOWSKI, R. F. & SCHNEIDER, M. F. (1976). A non-linear voltage dependent charge movement in frog skeletal muscle. *J. Physiol.* **254**, 245–284.

COHEN, L. B., HILLE, B. & KEYNES, R. D. (1970). Changes in axon birefringence during the action potential. *J. Physiol.* **211**, 495–515.

COHEN, L. B., HILLE, B., KEYNES, R. D., LANDOWNE, D. & ROJAS, E. (1971). Analysis of the potential-dependent changes in optical retardation in the squid giant axon. *J. Physiol.* **218**, 205–237.

COHEN, L. B., SALZBERG, B. M., DAVILA, H. V., ROSS, W. N., LANDOWNE, D., WAGGONER, A. S. & WANG, C. H. (1974). Changes in axon fluorescence during activity: molecular probes of membrane potential. *J. Membrane Biol.* **19**, 1–36.

COLBY, R. H. (1971). Intrinsic birefringence of glycerinated myofibrils. *J. cell Biol.* **51**, 763–771.

DULHUNTY, A. F. & FRANZINI-ARMSTRONG, G. (1975). The relative contributions of the folds and caveolae to the surface membrane of frog skeletal muscle fibres at different sarcomere lengths. *J. Physiol.* **250**, 513–539.

EBERSTEIN, A. & ROSENFALCK, A. (1963). Birefringence of isolated muscle fibres in twitch and tetanus. *Acta physiol. scand.* **57**, 144–166.

FRANZINI-ARMSTRONG, C. (1970). Studies of the triad. I. Structure of the junction in frog twitch fibres. *J. cell. Biol.* **47**, 488–499.

FRANZINI-ARMSTRONG, C. (1973). Membrane systems in muscle fibres. In *The Structure and Function of Muscle*, vol. 1, ed. BOURNE, G. H., pp. 532–617. New York: Academic Press.

HUXLEY, H. E. & HANSON, J. (1957). Quantitative studies on the structure of cross-striated myofibrils. I. Investigations by interference microscopy. *Biochim. biophys. Acta* **23**, 229–249.

MOBLEY, B. A. & EISENBERG, BRENDA R. (1975). Sizes of components in frog skeletal muscle measured by methods of stereology. *J. gen. Physiol.* **66**, 31–45.

OETLIKER, H., BAYLOR, S. M. & CHANDLER, W. K. (1975). Simultaneous changes in fluorescence and optical retardation in single muscle fibres during activity. *Nature, Lond.* **257**, 693–696.

PEACHEY, L. D. (1965). The sarcoplasmic reticulum and transverse tubules of frog's sartorius. *J. cell Biol.* **25**, 209–231.

PEACHEY, L. D. & SCHILD, R. F. (1968). The distribution of the T-system along the sarcomeres of frog and toad sartorius muscles. *J. Physiol.* **194**, 249–258.

RAYNS, D. G., SIMPSON, F. O. & BERTAUD, W. S. (1968). Surface features of striated muscle. II. Guinea-pig skeletal muscle. *J. cell Sci.* **3**, 475–482.

SANDOW, A. (1966). Latency relaxation: A brief analytical review. *M.C.V. Quarterly* **2**, 82–89.

SCHNEIDER, M. F. & CHANDLER, W. K. (1973). Voltage dependent charge movement in skeletal muscle: a possible step in excitation–contraction coupling. *Nature, Lond.* **242**, 244–246.

VON MURALT, A. (1932). Über das Verhalten der Doppelbrechung des quergestreiften Muskels während der Kontraktion. *Pflügers Arch. ges Physiol.* **230**, 299–326.

VON MURALT, A. (1975). The optical spike. *Phil. Trans. R. Soc.* B **270**, 411–423.

ZAMPIGHI, E. G., VERGARA, J. & RAMON, F. (1975). The connection between the T-tubules and the plasma membrane in frog skeletal muscle. *J. cell Biol.* **64**, 734–740.